

INDUCTION OF URINARY BLADDER PATHOLOGY IN MALE AND FEMALE C3H MICE EXPOSED TO SODIUM ARSENITE FROM GESTATION THROUGH YOUNG ADULthood

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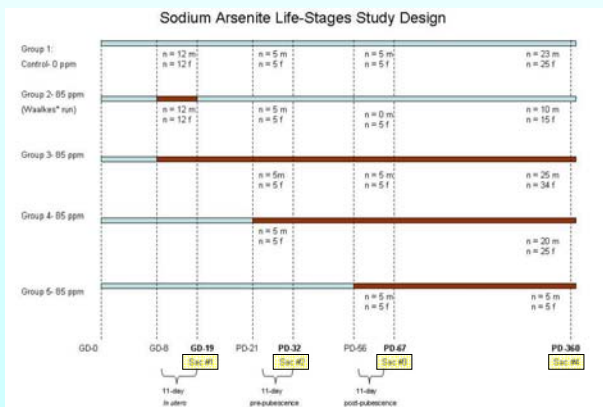
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Objectives

- Determine the impact/susceptibilities of sodium arsenite on tumor induction at various life-stages (gestation, pre-pubescence, and post-pubescence)
- Determine molecular biomarkers predictive of age-related susceptibility to chemical carcinogenesis through differential gene and protein expression.
- Using this life-stage data, more accurately determine environmental exposure standards for arsenic that do not rely on default correction factors.

Introduction

Epidemiology studies suggest that chronic exposure to inorganic arsenic is associated with cancer of the skin, urinary bladder and lung as well as the kidney and liver. Recently, an *in utero* animal model was developed to characterize the carcinogenic properties of inorganic arsenic (Waalkes et al., 2003). A brief exposure to sodium arsenite *in utero* (gestation day 8 to 18, GD8-18) increased liver, lung, adrenal, ovarian and uterine tumors in C3H and/or CD-1 mice.



This research study is designed to fill known data gaps associated with arsenic cancer risk, particularly the influence of age on cancer susceptibility from arsenic exposure. To do this, C3H mice were exposed to 85 ppm inorganic arsenic in their drinking water for 11 days during three stages of development: *in utero* (gestation day 8–19), pre-pubescence (postnatal day 21–32), and post-pubescence (postnatal day 56–67). Target tissues were taken and analyzed for pathology changes at PD67 and PD360. Additional tissue was fractionated for analysis of protein profiles by 2-D gel electrophoresis (DIGE) and mass spectrometry and gene regulation by microarray and DNA methylation analyses.

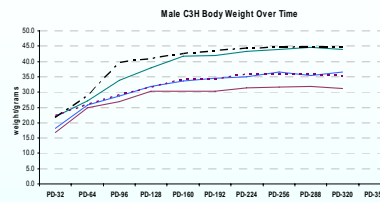


Table 1. Male C3H Pathology Incidence Summary (PD-67)

	Group 1	Group 2	Group 3	Group 4	Group 5
Bladder					
Eosinophilic inclusions	0/5	nd	5/5	nd	5/5
Lymphoid Aggregate	0/5	nd	0/5	nd	0/5
Adrenals					
X-zone Regression	0/5	nd	0/5	nd	0/5

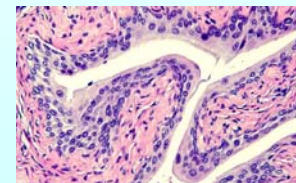


Figure 1: Control urinary bladder with normal transitional epithelium, two to three cell layers thick

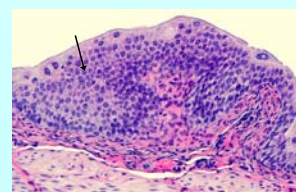


Figure 3: Urinary bladder from sodium arsenite treated mouse bladder, demonstrating thickened transitional epithelium

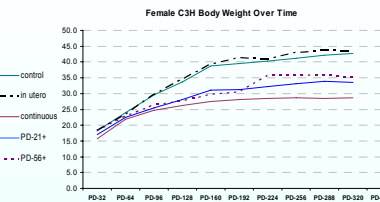


Table 2. Female C3H Pathology Incidence Summary (PD-67)

	Group 1	Group 2	Group 3	Group 4	Group 5
Bladder					
Eosinophilic inclusions	0/5	1/5	5/5	nd	5/5
Lymphoid Aggregate	0/5	0/5	0/5	nd	2/5
Adrenals					
X-zone Regression	0/5	0/5	2/5	nd	0/5
Uterus					
Hyperplasia	0/5	0/5	2/5	nd	1/5

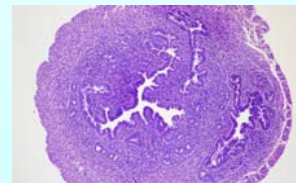


Figure 2: Uterus from arsenite treated mouse demonstrating endometrial hyperplasia

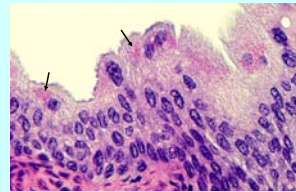


Figure 4: Urinary bladder from sodium arsenite treated mouse bladder, demonstrating intracytoplasmic eosinophilic inclusions.

Observations

Body Weight: Through PD-64, no significant body weight differences were observed. By PD-96, the continuously dosed mice (group 3) were significantly lighter than the control (group 1) and *in utero* (group 2) exposed mice. By PD-128, pre- and post-pubescence exposed mice (group 4 and 5, respectively) also weighed significantly less than the control and *in utero* exposed mice ($p \leq 0.05$).

Food and Water Consumption: Prior to PD-96, dosed mice (groups 3-5) consumed less water than the control (group 1) or *in utero* (group 2) exposed mice. Following this time point, when dividing mouse weight by ml of water consumed, there was no significant difference observed in water consumption ($p \leq 0.05$). There was no significant difference in feed consumption prior to PD-96. However, on a corrected ratio (grams body wt/grams feed consumed) groups 3-5 consumed significantly more feed than the controls and *in utero* exposed (data not shown, $p \leq 0.05$).

Bladder: At PD-67, there were no observable signs of urinary bladder pathology in the control mice. One *in utero* exposed female mouse exhibited eosinophilic inclusions. All other treated mice (male and female) from groups 3 and 5 exhibited eosinophilic inclusions at PD-67. Two females from group 5 also exhibited lymphoid aggregates.

Adrenal: Two female mice from the continuously dosed group (group 3) exhibited x-zone regression of the adrenals. No adrenal x-zone regression was observed in male mice or female mice from the control (group 1) and other treatment groups (group 2 and 5).

Uterus: No observable uterine hyperplasia were observed in the control (group 1) or *in utero* exposed (group 2). However, two (of five) mice exhibited uterine hyperplasia in the continuously dosed mice (group 3) and one (out of five) from post-pubescence exposed (group 5).

NOTE: Due to limited offspring, group 4 was not evaluated at this time point (PD-67).

Conclusion

These results demonstrate that a brief exposure, *in utero* or post-pubescence, to sodium arsenite is sufficient in inducing aberrant changes in urinary bladder and/or uterine pathology in mice. Prolonged arsenite exposure from gestation through young adulthood increases the severity/incidence of these lesions

On-Going Biological Endpoints Analysis: Tissues collected at GD-19, PD-32, and PD-67 is undergoing genomic and proteomic profile evaluation. Affymetrix Mouse Genome 430A 2.0 arrays was used to evaluate differential gene expression and 2-D DIGE with LC/MS/MS will be used to identify differentially expressed proteins.

Tissues collected at PD-360 will also undergo histopathological evaluation for preneoplastic and neoplastic lesions. Tumor incidence, latency and multiplicity parameters will be evaluated in all groups after 360 days of 85 ppm sodium arsenite exposure.

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